General Disclaimer

One or more of the Following Statements may affect this Document

- This document has been reproduced from the best copy furnished by the organizational source. It is being released in the interest of making available as much information as possible.

- This document may contain data, which exceeds the sheet parameters. It was furnished in this condition by the organizational source and is the best copy available.

- This document may contain tone-on-tone or color graphs, charts and/or pictures, which have been reproduced in black and white.

- This document is paginated as submitted by the original source.

- Portions of this document are not fully legible due to the historical nature of some of the material. However, it is the best reproduction available from the original submission.

Produced by the NASA Center for Aerospace Information (CASI)

CR-171 802
C.1

"BIOCIDAL QUATERNARY AMMONIUM RESIN"

FINAL REPORT

submitted
by
GILBERT E. JANAUER
November 1983

(NASA-CR-171802) BIOCIDAL QUATERNARY AMMONIUM RESIN Final Report (State Univ. of New York) 33 p HC A03/MF A01 CSCL 06A

N84-29433

Unclas
G3/51 19884

Work Performed Under Contract No. NAS 9-16604

by
Gilbert E. Janauer
Professor of Chemistry
SUNY-Binghamton
Binghamton, New York 13901

for
NASA LYNDON B. JOHNSON SPACE CENTER
HOUSTON, TX 77058

TABLE OF CONTENTS

TABLE OF CONTENTS (cont.)

I. INTRODUCTION

Activated carbon (charcoal) and polymeric resin sorbents are widely used in the filtering/treatment of drinking water, mainly to remove dissolved organic and inorganic impurities and to improve the taste. Earlier hopes that activated carbon might "disinfect" water proved to be unfounded. Various microorganisms are eager to colonize and proliferate on sorbent beds causing what is known as microbial fouling. Although most microbes commonly found in filter beds are harmless nuisance bacteria, some species - e.g., Pseudomonas aeruginosa - can act as opportunistic pathogens.

Potential problems due to microbial infection of charcoal (or other sorbent) beds may be summarized as (a) a potential health risk to humans ingesting water from such filters, (b) plugging or clogging of columns due to excessive overgrowth of microorganisms, and (c) decrease of useful sorbent capacity (and lifetime) of the filter bed by rendering ineffective or inaccessible fouled sorption sites otherwise available for the removal of undesirable solute species.

The general objective of the research effort was to demonstrate the feasibility of protecting against microbial infestation charcoal or resin beds such as those to be incorporated into total water reuse systems in spacecraft. A logical choice for a disinfectant appeared to be a specialty ion exchange material first prepared in the laboratory of the principal investigator and found effective against various microorganisms (Janauer et al., Chapter 23, Vol. 1, "Chemistry In Water Reuse", J.W. Cooper, Ed., Ann Arbor Sci. Publ., 1981, pp. 501-522). These resins kill bacteria on contact and do not add toxic impurities to the treated water. Therefore, the bulk of experiments was carried out with these "Insoluble Polymeric

Contact Disinfectants" (IPCD) in combination with a representative charcoal. In addition to demonstrating (in principle) the feasibility of protecting charcoal (or ion exchange) beds by means of these biocidal quaternary ammonium resins, a number of specific research objectives were pursued through performing the project tasks outlined below. During the initial stages of the project the PI became aware of the existence of a surface bonding antimicrobial (Dow Corning 5700) which - like the insoluble polymeric contact disinfectants invented here (U.S. Patent 4,349,646, Sept. 14, 1982) - is also based on a quaternary ammonium function as the active group. Because of the reportedly good performance as a contact inhibitor of microorganisms (Isquith et al., Appl. Microbiology, 24, 859, 1972), this material was included in the study, but unfortunately did not live up to expectations.

II. TASKS PERFORMED

As stated in "Exhibit A" (statement of work) of the original document (Section 4., Contractor Tasks) the PI and his research group have addressed the following specific tasks in this investigation.

1. Different configurations for sorbent/IPCD beds were tested for their efficacy.
2. The composition required of protected beds so as to provide microbial control within such beds was ascertained.
3. Test series were conducted to subsequently establish the effects of influent pH, temp., concentration of iodide, and microbes upon disinfectant capacity of protectant beds; in particular, the effects of urea and sodium chloride concentration.
4. The results of small-scale laboratory tests were evaluated to estimate an optimum protected bed configuration and composition, capable of preventing microbial growth within beds.
5. Results of the work completed are described and discussed in this FINAL REPORT, as stipulated.

The proposed tests were done in small laboratory columns packed with IPCD/charcoal or resin or either material by itself (efficacy control tests) with suspensions of Escherichia coli and other microorganisms. System parameters were varied and disinfection by candidate IPCD resulting in prevention of microbial growth in "protected" beds followed by standard plate count technique (SPC) performed on treated effluents. Some in-bed tests and tests with an alternate, surface-bonding, disinfectant were added to the array of tasks during the initial stages of the project.

III. SUMMARY OF SIGNIFICANT RESULTS

1. Tests with candidate IPCD materials (without charcoal beds present) showed excellent efficacy against suspensions of 10^6 cells/ml of E. coli and Ps. aeruginosa at room temperature and low temperature in continuous and interrupted flow over a number of days (quaternaries are known to disinfect better at higher temperatures).

2. Among the three IPCD/charcoal layer-protection configurations considered, overbed and sandwichbed configurations (OBC, SBC) proved very effective during challenges with bacterial suspensions of 10^4-10^6 CFU/mL. (all experiments used 8mm I.D. glass columns)

3. The total packed bed OBC composition found effective contained a top layer of IPCD 2-4 cm thickness above a charcoal bed of 8-10 cm height. In SBC there was also a bottom layer of 2-4 cm of IPCD. Its only function was seen as disinfecting any microbes initially present in charcoal (if not completely sterilized before use), but SBC would be needed in situations where flow reversal could occur.

4. Total disinfection of suspensions of E. coli, B. subtilis, S. marcescens, S. faecalis and Ps. aeruginosa was achieved singly and in mixtures.

5. The presence of urea, ethanol, lactic acid, and sodium chloride did not adversely affect IPCD protectant action. (No pH studies were carried out, because in another study IPCD were shown effective from $pH \geq 5$ to $pH \leq 9$.)

6. Tests with an ostensibly promising surface-bonding commercial disinfectant (Dow Corning 5700) - carried out in addition to stated tasks - showed no measurable attenuation of viable cell count upon contacting bacteria suspensions with treated glass beads under a variety of conditions.

IV. DISCUSSION OF RESULTS

Results of completed, representative experiments are presented below. All column experiments to this date were run with 7 mm inside diameter glass columns equipped with frits (see Fig.I) as dividers between the charcoal and IPCD beds. Gravity flow feeding with frequent periodical monitoring of flow rate was used over the period covered. The computer actuated multichannel pump mentioned in the interim report (2/15/83) was completed, but due to delays in obtaining special, compatible tubing it was not used in the investigations described. These pumps will be used in most experiments proposed for a follow-up study. (see Fig. II)

Most procedures were routine, described in standard texts, except for the Dow Corning 5700 Antimicrobial study (see IV.1.A. below) and the synthesis of IPCD resins. The latter were prepared by amination/quaternization of 2% DVB-crosslinked, low capacity chloromethylated polystyrene followed by repeated wash cycles (acid/base/water) to achieve complete purity (see, e.g., I.H. Walfish and G.E. Janauer, Water, Air, Soil Pollution, 12, 477-484 (1979)). (Some modifications in the procedure were introduced for preparing larger batches and for making mixed functionality IPCD). The activated carbon used was identical to that used in earlier studies by the researchers of NASA at the Johnson Space Center (Barneby-Cheney type 165 activated carbon). All other materials were standard materials including convential anion exchange resins and reagent grade chemicals.

In order to give a better overview, the results obtained with single materials (i.e., results from experiments not carried out with protected beds) are presented in Section (IV. 1.) and the re-

sults of UBC, OBC, and SBC runs in Section IV.2.

1. Results with Single Materials

A. Investigation of Dow 5700 "Antimicrobial Agent"

Dow-Corning 5700 "Antimicrobial Agent" had been reported to be very effective as a protectant for surfaces against microbial growth. Its antimicrobial capability is based on the presence of a silicon/quaternary ammonium group bonded to the various surfaces. This material (henceforth "Si-quat") was applied to glass wool and glass beads of varying diameter (40 μ, 100 μ, and 4 mm) by several methods. The method most often used consisted of preparing a 1% solution of Dow 5700 in doubly distilled water. The glass beads were added to this solution and remained in contact for five minutes with constant stirring. After five minutes the solution was suction filtered off through a fine porosity fritted glass funnel. The beads were then dried. Drying was also done in several different ways including room temperaure, 100°C, and 150°C with glass beads and glass wool. Procedures used were those recommended by the manufacturer.

All results for column tests using glass beads and glass wool indicated that the treated materials showed little or no disinfectant capability against E. coli. Batch testing of these treated materials also proved unsuccessful.

Samples of all treated beads were tested by a visual/colorimetric method provided by Dow-Corning. Although many samples were tested positive (meaning the surface was indeed "treated") none of the batches showed any noticeable effect on E. coli in aqueous media. One might speculate that the Dow-Corning 5700 was only inhibitory and not -cidal on the glass surface or that

the glass bead surfaces did not carry a high enough concentration of disinfectant to effect water disinfection within the column contact time. Since no promising results were obtained in a number of trials (carried out in consultation with Dow-Corning workers) investigations using Si-quat were suspended.

B. Experiments Using Activated Carbon Only

Experiments using beds of activated carbon only were always necessary as control experiments. The typical column run was done with three grams of activated charcoal (washed and autoclaved) in a 8 mm glass column. Three grams of charcoal was chosen since it has been the amount used in most investigations involving IPCD. Table I shows data obtained from three identical columns. One notes the sorption effect of the activated carbon for the bacteria. Although the data are useful and show rapid adsorption of the microorganisms by the carbon, the actual numbers obtained are useful for comparison purposes only, not as quantitative determinations. Flow rate must be considered a crucial factor in these experiments, because . . at a high enough flow rate, increased shear forces within the column would tend to decrease adsorption of microorganisms. (The flow rates of 2.8, 3.2, and 4.0 mL/min for the three columns are comparable to those used in work with the IPCD.)

C. Disinfection Experiments with IPCD Resins

A considerable amount of work was done with newly synthesized IPCD resins as such (no charcoal present), to ensure resin performance prior to experiments with over-bed, under-bed, and sandwich-bed configurations (OBC, UBC, SBC) activated carbon assemblies. Representative data are given in Tables II

and III for a standard run, a run at low temperature, and an experiment in which IPCD was used to first disinfect E. coli suspensions and then Ps. aeruginosa (same columns, interrupted flow). E. coli and Ps. aeruginosa were chosen as target organisms for these resin "quality control" tests because E. coli and Ps. aeruginosa were organisms of interest in later experiments, because of their ubiquity in water, and tendency to colonize charcoal beds. Typical concentrations of organisms used in these tests were on the order of 10^6 CFU/mL.

D. Adsorption of Bacteria on Conventional Anion Exchange Resins

Results of a typical run with conventional ion exchange resinare presented to illustrate that such resins are strong sorbents (see also S. Daniels, in "Adsorption of Microorganisms to Surfaces", Chapt. 2, John Wiley & Sons, N.Y. 1980, pp. 7-58) for our test species. Table IV shows indeed that E. coli are strongly adsorbed by a Dowex 1 resin. This was shown in another study with both anion and cation exchange resins. Although strong sorption occurs there is always some viable cell count in effluents after a larger volume has been passed and the adsorption is completely reversible, i.e., live viable cells are released, when the bed is treated with electrolyte solution. This is not new information, but is important from the point of view of using ion exchange beds (e.g., in form of demineralizing units) in water reuse systems such as the systems considered for manned spacecraft. Thus, ion exchange beds have the ability to "preconcentrate" bacteria. However, since bacteria rapidly reach a "breakthrough" point, it will be necessary to protect ion exchange beds as well as charcoal beds

by IPCD. Without the benefit of this protection ion exchange beds colonized by bacteria may suffer severe decreases in the effective (dynamic) exchange capacity.

2. Investigations Using IPCD in OBC, SBC, and UBC

Most of the reported work has been done using IPCD as the solid contact disinfecting agent. As mentioned in the abstract of this report, OBC and SBC were used in later investigations after preliminary work showed that these were more effective than the UBC. In retrospect the UBC was not practical, because the idea is protection of the charcoal bed; by placing the IPCD resin bed after the charcoal bed this is not the case.

A. Disinfection of Escherichia coli by SBC, OBC and UBC

The results of a typical experiment using UBC, OBC and SBC are given in Tables V and VI. Table V shows the results of a five day experiment during which various solutions including 250 ml of Escherichia coli suspension at a concentration of approximately 1.5×10^6 CFU/mL were passed through the composite beds. Table VI shows the results of a similar investigation in which E. coli suspension was again passed through columns in the OBC and SBC configurations. This experiment was different in that a 100 ml volume was first applied, and the columns were then allowed to stand overnight. This procedure was repeated each day for the duration of the run. Unfortunately air bubbles formed after the third day and precluded further use of these columns. As mentioned previously the OBC and SBC performed well while the results using UBC were less satisfactory. One can assume that a rapid build-up of bacteria in the charcoal bed above the IPCD resulted in the failure of the UBC.

The results presented in Table VI show that flow interruption in presenting influent to the columns had no adverse effect. These results are typical for the effectiveness of OBC and SFJ in similar runs. A 24-hour run is shown in Table IX.

C. Disinfection of Mixed Bacterial Suspensions

The IPCD used in these investigations was shown to be effective against S. faecalis, E. coli, B. subtilis, S. marcescens, and Ps. aeruginosa. Some of these were disinfected singly, others in mixed suspensions with other bacteria. In one experiment a mixture of S. marcescens, E. coli, and S. faecalis was passed through columns in the OBC. In no one case was any significant growth found on the sample plates. The results for this experiment are given in Table VII. It is known that in the presence of E. coli the growth of S. marcescens is severely suppressed, and although present in the mixture of microorganisms, S. marcescens comprised a very small percentage of the total count of microorganisms.

D. Disinfection in the Presence of Organic Compounds and Electrolytes.

Several test series were carried out using various substances present in the influent water stream. Most of these were carried out using the OBC configuration. One experiment of interest was carried out over a period of several weeks. The first part of the experiment consisted of a simple "conditioning" of the columns using E. coli. The second part consisted of a challenge to the columns using B. subtilis and E. coli as target microorganisms in an influent water stream

containing urea, ethanol, sodium chloride, lactic acid, and iodide/triiodide. The third part of the experiment consisted of passing through a solution containing B. subtilis in the presence of a high concentration of organic and inorganic additives listed above. Unfortunately, further work with these columns was precluded due to the formation of air pockets in the column beds. The results of this experiment are given in table VIII.

V. PROBLEMS AND REMEDIES

Aside from the fact that the - principally very promising - Dow Corning 5700 surface bonding material did not disinfect when applied under conditions relevant to the objectives of this study, no major difficulties arose in most experiments, although some delays were encountered due to minor problems. One time-consuming problem during the beginning of the study concerned the seemingly trivial procedure of packing the minicolumns with completely air-free charcoal. Actually, quite a few experiments had to be aborted, because of leaks in connections, channeling, or air bubbles from charcoal not sufficiently deaerated, until the operator overcame this problem after a number of unsuccessful attempts. It was found that simply autoclaving the charcoal in water (rather than dry) and packing the column with a slurry and frequent addition of boiled water resulted in uniform packing and avoided the release of air from the charcoal. The necessity of using tubing free of microorganisms imposed special care, also.

Another problem was encountered when it was tried to sample directly from the interior of packed columns (before and after actual runs). The glass columns happened to be of a type that fractured when one used a glass cutter to create a "window" for sampling. The use of polymer tubing was considered, but then another way was found in that surface disinfected tygon tubing (ethanol washed) was put tightly over the glass columns, a small "window" cut into the tygon, and finally a hole created by a short blow with a small, flame treated metal tool. This way the column was held in place, even if fractured in one or two

spots. As it turned out, no important information was gleaned from these experiments, because from IPCD protected bed packings no colony forming units could be obtained, except when the activated carbon had not been properly sterilized (autoclaved). It is anticipated that the situation may be different with ion exchange resin packed columns, because of their much greater sorption tendency for bacteria. Thus, it may well be expected to "see" the bacteria migrate through resin beds (like chromatographic fronts). It will then be of advantage to perform more such experiments using the column-in-tube sampling "window" procedure.

Finally, it should be mentioned once again, that the PI had underestimated the time/effort actually spent on routine microbiological procedures. Bacteria need considerable time to grow, and all testing must take this basic fact into account. There is no remedy, just patience and much more effort than in analogous chemical systems. Because of this and the need to perform additional experiments with B. subtilis, the PI requested a no-cost extension which was very fruitful and in some ways advanced the project beyond initially set targets.

VI. SUGGESTED FUTURE WORK

The feasibility of protecting sorbent beds against microbial colonization by various representative bacteria in waters containing organics and inorganics has been demonstrated in principle. Some further work is needed with charcoal, but particularly, with ion exchange resins - strong reversible sorbents for live microorganisms - because of the necessity to preserve effective capacity and unobstructed flow in demineralizing units (DMU) required in closed water reuse systems. The following results would be required in developing the experimental basis for a protected water demineralization unit.

1. Sufficient data on the extent and rate of colonization (by representative test organisms) of convential ion exchange resins (anion & cation exchange resins both as such and in mixed beds as used in water demineralization). The loss of capacity and potential flow restriction due to fouling could be limiting factors in a real life situation.
2. In addition to OBC and SBC (both effective) configurations the potential ability of intermixed bed configuration (IBC) needs to be explored, particularly, in conjunction with ion exchange beds. (Stratification would not be expected in that case). Challenges with microorganisms in the presence of moderate to high solute concentrations would also be necessary to establish the "real world" efficacy.
3. Optimum configuration/composition of protected sorbent beds to be established by means of an efficient, statistically responsive experimental design matrix (low level resolution with

some confounding).

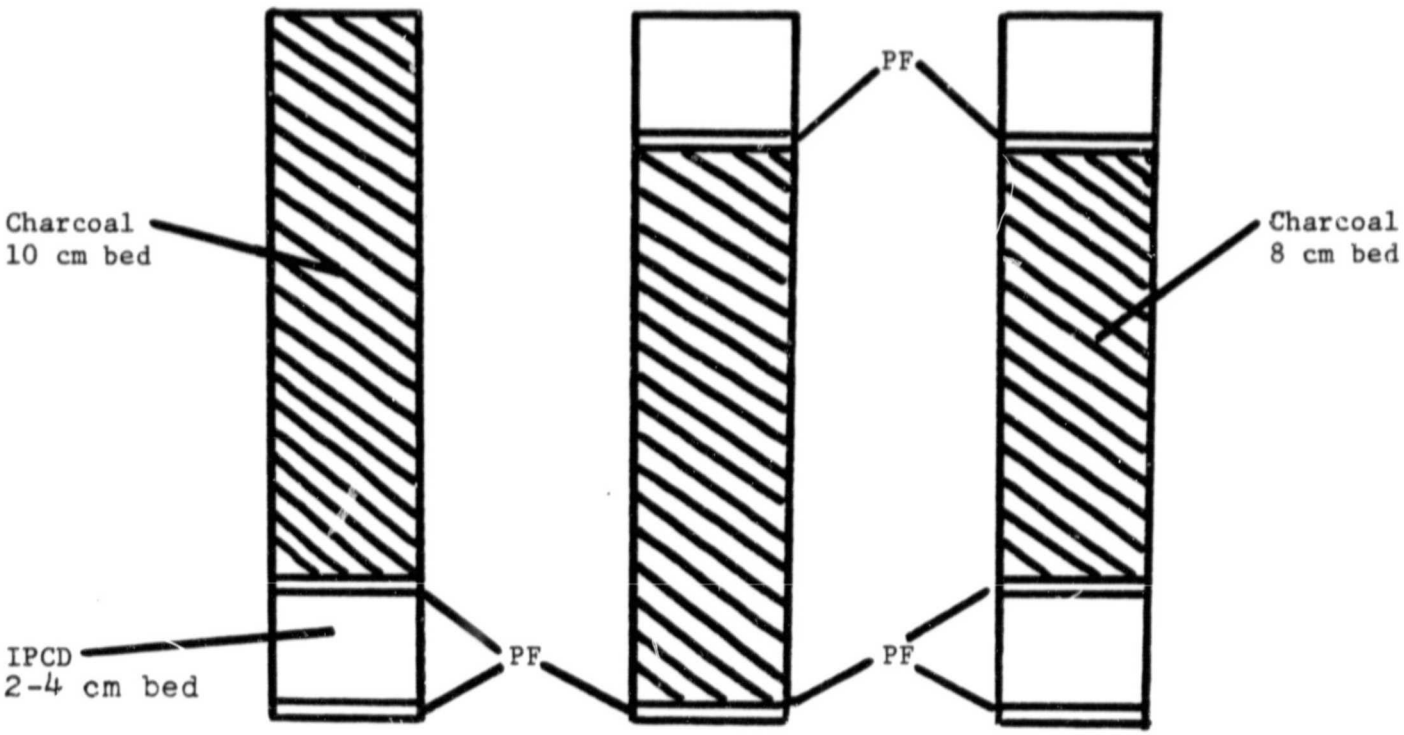
4. Total disinfection capacities at realistic flow rates determined for "optimum" IPCD-sorbent configuration/composition assemblies. Maximum permissible flow rates (to viable cell breakthrough) to establish system limitations under combined microbiological and chemical stress.

5. Modeling of preprototype DMU to be followed by development of prototype jointly with qualified engineering expert collaborating.

ORIGINAL PAGE IS
OF POOR QUALITY

Figure I

SCHEMATIC REPRESENTATION OF
TESTED COLUMN CONFIGURATIONS
(not drawn to scale)



UBC - Underbed Configuration

OBC - Overbed Configuration

SBC - Sandwich Bed Configuration

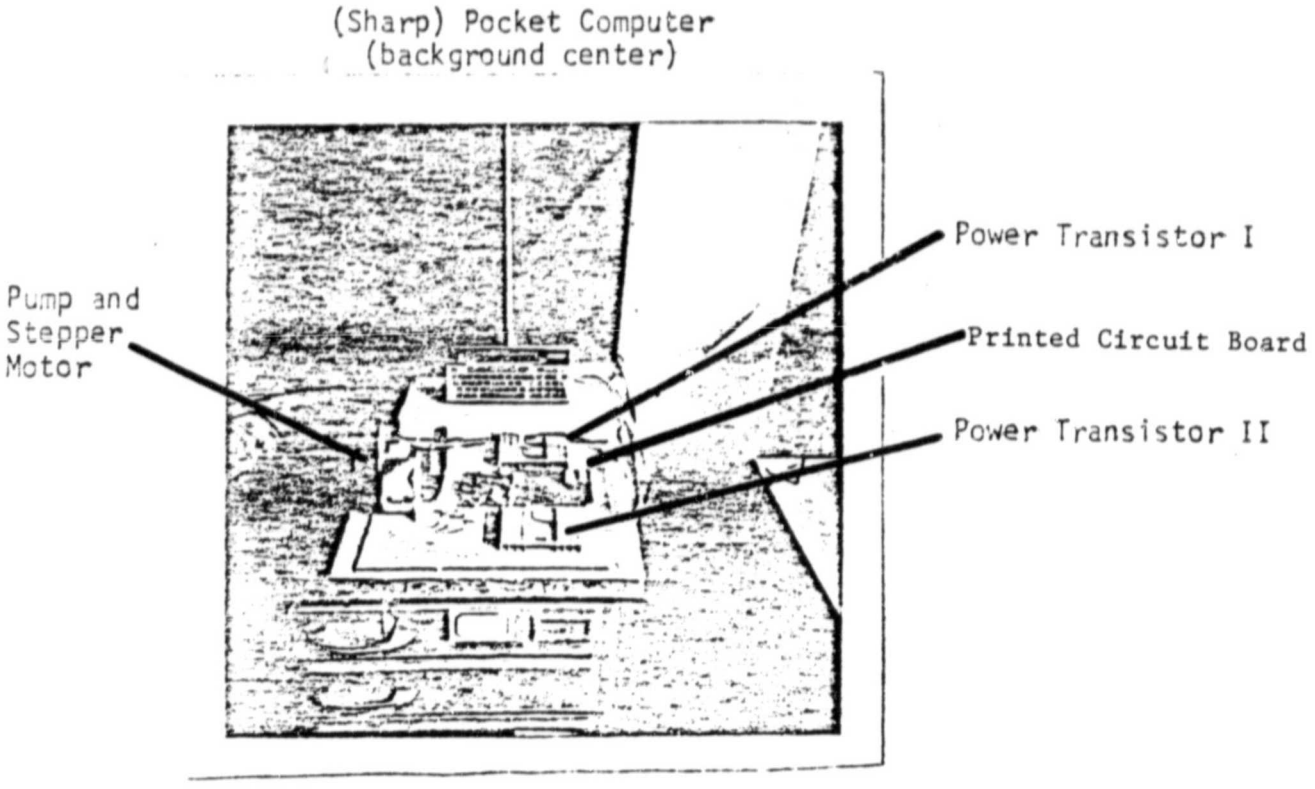
Charcoal - Washed Barnebey - Cheney type 165

PF - Polyurethane Frits (fine porosity , 40μ)

ORIGINAL PAGE IS
OF POOR QUALITY

Figure II

MOCK-UP OF POCKET COMPUTER-ACTIVATED PUMPING UNIT



One pocket computer can activate simultaneously up to 6 liquid metering systems at different rates of delivery (0.1 ml/min to 50 ml/min), except that limited memory does not permit all combinations of pulse durations.

Table I

ADSORPTION OF *E. COLI* FROM .01 M THAM*** BUFFER BY ACTIVATED CHARCOAL BEDS

Column 1

Flow Interval	Plate Counts**			
	1 mL	10^2	10^3	10^4
50	TNTC*	45	3*	1*
100	TNTC	140	14*	0
150	TNTC	262	21*	2*
200	TNTC	142	6*	0
250	TNTC	209	26*	2*
Column 2				
50	TNTC	202	9*	3*
100	TNTC	75	1*	0
150	TNTC	190	2*	2*
200	TNTC	204	21*	5*
250	TNTC	TNTC	33	5*
Column 3				
50	TNTC	134	4*	0
100	TNTC	TNTC	19*	2*
150	TNTC	173	7*	0
200	TNTC	220	13*	1*
250	TNTC	TNTC	42	7*

Column	Flow Rate (mL/min)
1	2.8
2	3.5
3	4.0

*** THAM is tris-hydroxyaminomethane in water buffered to a pH of 7.6.

** CFU per plate from serial dilutions

*Standard microbiological protocol states that plate counts below 30 are not statistically significant. Counts above 300 are "too numerous to count" (TNTC).

...ITY

Table II

REPRESENTATIVE DISINFECTION OF *E. COLI* SUSPENSION ON IPCD BED

Columns 1,2,3: each contains a 2 mL bed of IPCD only
Flow Rate: 1-3 mL/min; flow interrupted for 4 hours after 600 mL sampling, and for 12 hours after 1000 mL sampling
Concentration of organisms in the influent: 1×10^6 mL^{-1}

Column Number	Flow Interval	Plate Counts**			
		1 mL	10^2	10^3	10^4
1	100 mL	1*	0	0	0
	200	0	38	0	0
	400	0	7*	40	0
	600	0	0	0	0
		(4 HOUR INTERRUPTION)			
	800	0	0	0	0
	1000	0	0	3*	0
		(12 HOUR INTERRUPTION)			
	1200	0	0	0	0
	1400	0	0	0	0
	1600	0	0	0	0
	1800	0	0	0	0
2	100 mL	0	0	0	0
	200	0	0	0	0
	400	0	0	0	0
	600	4*	0	0	0
		(4 HOUR INTERRUPTION)			
	800	0	0	0	0
	1000	0	0	0	0
		(12 HOUR INTERRUPTION)			
	1200	0	0	0	0
	1400	0	0	0	0
	1600	0	0	0	0
	1800	0	0	0	0
3	100 mL	0	2*	0	0
	200	0	8*	0	0
	400	0	0	0	0
	600	0	0	0	0
		(4 HOUR INTERRUPTION)			
	800	0	0	0	0
	1000	0	0	0	0
		(12 HOUR INTERRUPTION)			
	1200	0	0	0	16*
	1400	0	0	0	0
	1600	3*	0	0	0
	1800	7*	0	0	0

*Standard microbiological protocol states that plate counts below 30 are not statistically significant. Counts above 300 are "too numerous to count" (TNTC).

**CFU per plate from serial dilutions

ORIGINAL PAGE IS
OF POOR QUALITY

Table II (cont.)

DISINFECTION OF E. COLI SUSPENSION AT 25°C AND 3°C ON IPCD BED

Flow Rate: 2-3 mL/min; flow interrupted for 12 hours after the 400 mL sampling at 25°C, and for 72 hours after the 600 mL sampling at 3°C
Column 1: 2 mL bed of IPCD run at 25°C
Column 2: 2 mL bed of IPCD run at 3°C
Concentration of organisms at 25°C: 2×10^6 mL^{-1}
Concentration of organisms at 3°C: 2×10^6 mL^{-1}

Column Number	Flow Interval	Plate Counts** 1 mL	10^2	10^3	10^4
1 (25°C)	100 mL	1*	0	0	0
	200	0	0	0	0
	400	0	0	0	1*
		(12 HOUR INTERRUPTION)			
	500	0	0	0	1*
2 (3°C)***	100 mL	0	5*	0	0
	200	0	0	0	0
	400	1*	1*	0	0
	600	1*	0	0	0
		(72 HOUR INTERRUPTION)			
	800	0	6*	1*	0
	1000	0	0	0	0

*Standard microbiological protocol states that plate counts below 30 are not statistically significant. Counts above 300 are "too numerous to count" (TNTC).

**CFU per plate from serial dilutions

***The same column was used for the runs at 25°C and 3°C. The run at 3°C was carried out after the run at 25°C. Volumes shown at 3°C are in addition to those at 25°C.

Table III

DISINFECTION OF *E. COLI* AND *PSEUDOMONAS AERUGINOSA* BY IPCD BED IN SEQUENTIAL APPLICATIONS

Flow Rate: 3-4 mL/min; flow interrupted for 24 hours after 1000 mL sampling and agin for 12 hours after 1200 mL sampling
Column 1: 3 mL bed of IPCD
Concentration of organisms in the influent: 1×10^6 mL^{-1} (in this first part of the experiment, *E. coli* was target organism.

Column Number	Flow Interval	Plate Counts**			
		1 mL	10^2	10^3	10^4
1	100 mL	0	0	0	0
	200	0	0	0	0
	400	0	0	0	0
	600	0	0	0	0
	800	0	0	0	0
	1000	0	0	0	0
		(24 HOUR INTERRUPTION)			
	1200	0	0	2*	0
		(12 HOUR INTERRUPTION)			
	1400	9*	0	0	0
	1600	8*	0	0	0
	1800	9*	0	0	0
	2000	17*	0	0	0

*Plate counts below 30 are not significant, counts above 300 are "too numerous to count (TNTC).

**CFU per plate from serial dilutions

The column used in this experiment was used to disinfect *Ps. aeruginosa* 4 days later. The results follow on the next page.

ORIGINAL PAGE IS
OF POOR QUALITY

Table III (cont.)

DISINFECTION OF E. COLI AND PSEUDOMONAS AERUGINOSA BY IPCD BED IN SEQUENTIAL APPLICATIONS

Flow Rate: .2-1.0 mL/min; flow interrupted for 9 hours after the 600 mL sampling
Column 1: 3 mL bed of IPCD
Concentration of organisms in the influent: $1 \times 10^6 mL^{-1}$; in this part of the experiment Ps. aeruginosa was target organism

Column Number	Flow Interval	Plate Counts**			
		1 mL	10^2	10^3	10^4
1	100 mL	TNTC	4*	0	0
	200	TNTC	0	0	0
	400	TNTC*	11*	0	0
	600	TNTC	25*	23*	0
	775***	TNTC	TNTC	42	2*
	785	67	40	1*.	5*

*Plate counts below 30 are not statistically significant, above 300 are "too numerous to count" (TNTC).

**CFU per plate from serial dilutions

***This sample was taken immediately after resuming flow through the standing column. This may be viewed as a flow interruption test.

Table IV

ADSORPTION OF *E. COLI* ON COMMERCIAL ION EXCHANGE RESIN

Resin: Dowex 1X2 200-400 mesh Cl^- form

Flow Rate: 1-2 mL/min

Flow Interval	Plate Counts**			
	1 mL	10^2	10^3	10^4
50 mL	10*	0	1*	0
100 mL	10*	0	0	1*
250 mL	6*	0	0	1*

*Standard microbiological protocol states that plate counts below 30 are not statistically significant.

**CFU per plate from serial dilutions

Table V

DISINFECTION OF E. COLI USING SBC, OBC, AND UBC COLUMN ASSEMBLIES

Column 1: 3 grams of washed, autoclaved charcoal

Column 2: SBC configuration, 2 cm IPCD beds, 10 cm bed of charcoal

Column 3: UBC configuration, 4 cm IPCD bed, 10 cm bed of charcoal

Column 4: OBC configuration, 4 cm IPCD bed, 10 cm bed of charcoal

Flow rate for all columns: 2-2.5 mL/min
Bed diameter for all columns: 8mm
Concentration of organisms in the influent: 1×10^5 mL^{-1}

Day 1 Results

Column Number	Flow Interval	Plate Counts**			
		1 mL	10^2	10^3	10^4
1 (Control)	25 mL	TNTC*	1*	0	0
	50	TNTC	4*	0	0
	100	TNTC	6*	0	0
	250	TNTC	0	0	0
2 (SBC)	25 mL	0	0	0	0
	50	0	0	0	0
	100	0	0	0	0
	250	0	0	0	0
3 (UBC)	25 mL	[illegible]*	0	0	0
	50	45	0	0	0
	100	36	0	0	0
	250	76	0	1*	0
4 (OBC)	25 mL	0	2*	0	0
	50	0	0	0	0
	100	0	0	0	0
	250	3*	0	0	0

*Standard microbiological protocol states that plate counts below 30 are not statistically significant. Counts above 300 are "too numerous to count" (TNTC).

**CFU per plate from serial dilutions

Table V (cont.)

Flow rate for all columns: 2-2.5 mL/min
Bed diameters for all columns: 8mm
Concentration of organisms in the influent: 1×10^5 mL^{-1}

Day 3 Results

Column Number	Flow Interval***	Plate Counts**			
		1 mL	10^2	10^3	10^4
1 (Control)	1 mL	----	----	----	----
	25	49	0	0	1*
	50	----	----	----	----
2 (SBC)	1 mL	0	0	0	0
	25	0	0	0	0
	50	----	----	----	----
3 (UBC)	1 mL	TNTC*	11*	0	0
	25	19*	0	0	0
	50	----	----	----	----
4 (OBC)	1 mL	33	0	0	0
	25	4*	1*	0	0
	50	----	----	----	----

Day 5 Results

1 (Control)	1 mL	50
	50	12*
2 (SBC)****	1 mL	0
	50	0
3 (UBC)	1 mL	TNTC
	50	23*
4 (OBC)	1 mL	27*
	50	0

*Standard microbiological protocol states that plate counts below 30 are not statistically significant. Counts above 300 are "too numerous to count" (TNTC).

**CFU per plate from serial dilutions

***Samples were taken from the columns at the first mL to pass after standing for 48 hours, and after 25 and 50 mL of sterile buffer were passed through the columns respectively.

****Intracolumn samples from the charcoal bed of column 2 showed no growth.

Table VI

DISINFECTION OF E. COLI USING SBC AND OBC ASSEMBLIES

Column 1: Control, 3 grams of washed, autoclaved charcoal
Column 2: SBC, 3 cm resin beds, 2 grams of charcoal
Column 3: OBC, 6 cm resin bed, 3 grams of charcoal
Bed diameter: 8mm for all columns
Concentration of organisms in the influent: 2×10^6 mL^{-1}

Results

Column Number	Flow Interval	Plate Counts**			
		1 mL	10^2	10^3	10^4
1 (Control)	100 mL	132	3*	0	1*
	200	TNTC*	7*	1*	1*
	300	TNTC	TNTC	21*	18*
2 (SBC)	100 mL	0	0	0	0
	200	0	0	0	0
	300	0	0	0	1*
3 (OBC)	100 mL	0	0	0	0
	200	0	0	0	0
	300	0	0	0	0

*Standard microbiological protocol states that plate counts below 30 are not statistically significant. Counts above 300 are "too numerous too count" (TNTC).

**CFU per plate from serial dilutions

Table VII

DISINFECTION OF E. COLI, S. MARCESCENS, AND S. FAECALIS USING OBC ASSEMBLY

Column 1: OBC, 4 cm IPCD, 10 cm charcoal
Column 2: OBC, 4 cm sea sand, 10 cm charcoal (NO IPCD)
Column 3: OBC, 4 cm IPCD, 10 cm charcoal
Concentration of organisms in the influent: 2×10^5 mL^{-1}

Column Number	Flow Rate at Sample Interval***			
	0-25 mL	25-50 mL	50-100 mL	100-250 mL
1 (OBC)	.81	1.2	2.2	2.3
2 (OBC no IPCD)	.63	1.8	1.6	2.6
3 (OBC)	.76	1.1	1.5	2.5

Results

Column Number	Flow Interval	Plate Counts**			
		1 mL	10^2	10^3	10^4
1	25 mL	0	0	1*	1*
	50	0	0	0	0
	100	0	0	0	0
	250	0	0	1*	1*
2	25 mL	TNTC	TNTC*	45	5*
	50	TNTC	TNTC	102	6*
	100	TNTC	TNTC	202	9*
	250	TNTC	TNTC	170	2*
3	25 mL	0	0	0	0
	50	1*	0	0	0
	100	1*	0	0	0
	250	2*	0	0	0

*Standard microbiological protocol states that plate counts below 30 are not statistically significant. Counts above 300 are "too numerous to count" (TNTC).

**CFU per plate from serial dilutions

***Flow rates in mL/min

ORIGINAL PAGE IS
OF POOR QUALITY

Table VIII

DISINFECTION OF E. COLI
USING AN OBC COLUMN

Column 1: OBC, 4 cm IPCD, 10 cm charcoal
Column 2: 4 cm sea sand, 10 cm charcoal (NO IPCD)
Column 3: OBC, 4 cm IPCD, 10 cm charcoal
Bed Diameter: 8mm for all columns
Flow Rates: approximately 2 mL/min for all columns
Concentration of organisms in the influent: $2 \times 10^5 \cdot mL^{-1}$

Results: all plates on which samples from columns 1 and 3 were plated showed no growth after 500 mL of E. coli were passed through.

DISINFECTION OF A MIXTURE (E. COLI & B. SUBTILIS) OF MICROORGANISMS USING AN OBC COLUMN

The columns used in the above experiment were allowed to stand for five days and used again in this one. This part of the experiment consisted of passing through a mixture of microorganisms in a solution of doubly distilled water containing various additives. These additives were selected according to data furnished in the original proposal. The columns performed well throughout this experiment.

Concentration of organisms in the influent: 2×10^5 mL^{-1}

Column Number	Flow Rate at Sample Interval***		
	0-50 mL	50-100 mL	100-250 mL
1	1.6	1.6	1.4
2	1.2	1.0	1.0
3	1.1	1.3	1.1

Results

Column Number	Flow Interval	Plate counts**			
		1 mL	10^2	10^3	10^4
1	50 mL	0	0	0	0
	100	0	1*	0	0
	250	0	0	1*	1*
2	50 mL	----	----	----	----
	100	TNTC	19*	2*	0
	250	TNTC	12*	0	1*
3	50 mL	0	0	1*	1*
	100	1*	0	0	0
	250	0	0	1*	0

*Standard microbiological protocol states that plate counts below 30 are not statistically significant. Counts above 300 are "too numerous to count" (TNTC).

**CFU per plate from serial dilutions

***Flow rates in mL/min

Table VIII (cont.)

Additive	Concentration(ppm)
urea	75
ethanol	10
sodium chloride	45
lactic acid	35
iodide	5

Results

Column Number	Flow Interval	Plate Counts**		
		50 mL	100 mL	250 mL
1	1 mL	0	0	0
	10^2	0	0	0
	10^3	1*	0	1*
	10^4	1*	0	1*
2	1 mL			
	10^2	Did Not Run Due To System Leak		
	10^3			
	10^4			
3	1 mL	1*	1*	0
	10^2	0	0	0
	10^3	0	0	0
	10^4	0	0	0

In this third part of the experiment B. subtilis was used as the target microorganism, this experiment was run after allowing the columns to stand for two weeks.

Flow Rates: 2-2.5 mL/min for all columns
Concentration of organisms in the influent: 2×10^5 mL^{-1}

*Standard microbiological protocol states that plate counts below 30 are not statistically significant. Counts above 300 are "too numerous to count" (TNTC).

**CFU per plate from serial dilutions

Table IX

DISINFECTION OF E. COLI
IN TWENTY-FOUR HOUR NON-STOP
COLUMN OPERATION

Column 1: SBC, 4 cm IPCD beds, 6 cm charcoal bed
Column 2: OBC, 4 cm IPCD bed, 10 cm charcoal bed
Flow Rate: 1.7 mL/min for both columns
Bed Diameter: 8mm for each column
Concentration of organisms in the influent: 1×10^4 mL^{-1}

Results

Column Number	Flow Interval	Plate Counts**			
		1 mL	10^2	10^3	10^4
1	200 mL	0	0	0	0
	400	0	0	0	0
	600	0	0	0	0
	800	0	0	0	0
	1000	0	0	0	0
	1200	0	0	0	0
	1400	0	0	0	0
	1600	0	0	0	0
2	200 mL	0	0	0	1*
	400	0	0	0	0
	600	0	0	0	0
	800	0	0	0	0
	1000	0	0	0	0
	1200	0	0	0	0
	1400	0	0	0	0
	1600	0	0	0	0

*Standard microbiological protocol states that plate counts below 30 are not statistically significant. Counts above 300 are "too numerous to count" (TNTC).

**CFU per plate after serial dilutions